Genomic Amplification of the Human *Plakophilin 1* Gene and Detection of a New Mutation in Ectodermal Dysplasia/Skin Fragility Syndrome

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Ectodermal dysplasia/skin fragility syndrome is a recently described autosomal recessive disease affecting skin, nails, and hair (MIM 604536), that results from mutations in *plakophilin 1*, a structural component of desmosomes. We report a new *plakophilin 1* mutation in an affected patient as well as detailing the intron–exon organization of the gene to facilitate future polymerase chain reaction-based mutation screening. Using polymerase chain reaction amplification of genomic DNA, we identified 15 exons spanning approximately 50 kb. Direct sequencing disclosed several nonpathogenic intragenic polymorphisms, as well as a homozygous splice site mutation (1233–2 A→T; GenBank Z73678) in a 17 y old affected male. The clinical features comprised skin erosions, dystrophic nails, sparse hair, and painful thickening and cracking of palms and soles. Skin biopsy showed negative immunolabeling with an anti-*plakophilin 1* antibody and small desmosomes. These results expand the database of *plakophilin 1* mutations and demonstrate the importance of this protein in the stabilization of desmosomal adhesion in terminally differentiating keratinocytes. ***Key words: cell adhesion/desmosome/genodermatosis/keratinocyte. J Invest Dermatol 115:368–374, 2000***

Desmosomes are intercellular adhesive junctions found primarily in epithelial cells where they are involved in cell–cell communication as well as cell adhesion (Garrod, 1993, 1996; Kouklis *et al*, 1995; Green and Jones, 1996). The cell adhesion components of the desmosome are the desmosomal cadherins comprising the desmogleins (DSG 1–3) and the desmocollins (DSC 1–3). Proteins of the desmosomal plaque—desmoplakin, plakoglobin, and plakophilin 1—link the desmosomal cadherins to the keratins that make up the 10 nm intermediate filament network (Smith and Fuchs, 1998). Both plakoglobin and plakophilin 1 are members of a multigene family displaying homology to the armadillo signal transduction protein in *Drosophila* (Riggleman *et al*, 1990; Peifer *et al*, 1994). Armadillo-like proteins are structural proteins as well as signaling molecules consisting of globular amino- and carboxy-terminal domains that flank a central region of imperfect "arm" repeats 42–45 amino acids in length (Hatzfeld, 1999). Plakoglobin, armadillo, and β-catenin have the highest homology and contain 13 "arm" repeats (Cowin and Burke, 1996), whereas plakophilin 1 is in a subset of proteins containing 10 imperfect "arm" repeats, including plakophilin 2 (Mertens *et al*, 1996), plakophilin 3 (Schmidt *et al*, 1999), plakophilin 4/p0071 (Hatzfeld and Nachtsheim, 1996), δ-catenin-1/p120^{ctn} (Reynolds *et al*, 1989), δ-catenin-2/neural plakophilin-related armadillo-repeat protein (Paffenholz and Franke, 1997), and armadillo repeat gene deleted in velocardiofacial syndrome (Sirotkin *et al*, 1997). Although some of these armadillo proteins display high homology, their genes are dispersed throughout the genome (Bonne *et al*, 1998). Plakophilin 1 or "band 6 protein", first isolated from bovine muzzle desmosome fractions (Kapprell *et al*, 1988), is an 80.5 kDa positively charged, nonglycosylated cytokeratin binding protein (Hatzfeld *et al*, 1994). Recent cDNA cloning of both human (Heid *et al*, 1994) and bovine plakophilin 1 (Kapprell *et al*, 1988) has revealed a primary sequence consisting of a globular amino-terminal domain followed by 9.2 armadillo repeats that stretch to the carboxy terminus. Two human splice variants have been characterized both of which have at least two different polyadenylated forms (Schmidt *et al*, 1997). Plakophilin 1a consists of 726 amino acids and is a major constituent of the desmosomal plaque in stratified and complex epithelia, whereas plakophilin 1b consists of 747 amino acids and is located entirely in the nucleus (Schmidt *et al*, 1997). Plakophilin splice variants 1a and 1b are encoded by the same single copy gene mapped to 1q31.3–1q32.1 (Cowley *et al*, 1997; Bonne *et al*, 1998; Schmidt *et al*, 1999). Clues to the function of *PKP1* in epithelial biology were first established in reports of two unrelated children with a novel form of autosomal recessive skin disease, comprising skin fragility and ectodermal dysplasia associated with total ablation of *PKP1* (McGrath *et al*, 1997, 1999). To facilitate mutation detection in other patients with this genodermatosis we report the development of a strategy for the detection of sequence variants in the *PKP1* gene. Using this approach, we also report identification of a new *PKP1* mutation in a 17 y old male with ectodermal dysplasia/skin fragility syndrome. This individual represents the

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Abbreviations: PTC, premature termination codon; *PKP1*, plakophilin 1 gene.

oldest patient with *PKP1* ablation and the clinical features in this case provide further insights into the role of *PKP1* in epithelial adhesion, differentiation, and development.

MATERIALS AND METHODS

Characterization of plakophilin 1 genomic structure Using preliminary *PKP1* structure data (McGrath *et al*, 1999), primers based on the cDNA sequence of plakophilin 1a (GenBank accession no. Z73678) were used to amplify plakophilin 1 introns from control human genomic DNA (Roche Molecular Diagnostics, Lewes, U.K.). For introns 3–14 approximately 200 ng of genomic DNA was added to a premix containing polymerase chain reaction (PCR) buffer [67 mM Tris–HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.17 mg per ml bovine serum albumin (Sigma, Poole, U.K.), and 10 mM 2-mercaptoethanol], 10 nmol of each dNTP, 20 pmol of each primer in a total volume of 50 μl. After an initial denaturation at 95°C for 2 min, 2.5 units of Taq polymerase (Promega, Madison, WI) was added, followed by 35 cycles of 94°C for 10 s, annealing for 10 s, 72°C for 1 min, with a final incubation of 72°C for 5 min. For introns 1 and 2, approximately 200 ng of genomic DNA was added to a premix containing Expand Long 20 kb^+ buffer (Roche Molecular Diagnostics), 25 nmol of each dNTP, 4% dimethyl sulfoxide (Sigma), and 20 pmol of each primer in a total volume of 50 μl. After an initial denaturation at 92°C for 2 min, 2.5 units of Expand Long 20 kb^+ enzyme mixture was added (Roche Molecular Diagnostics), followed by 35 cycles of 92°C for 10 s, annealing for 10 s, 68°C for 3 min (intron 1) or 68°C for 14 min (intron 2), with a final incubation of 68°C for 5 min. The PCR products were examined by 1% agarose gel electrophoresis, purified using spin columns (Qiagen, Crawley, U.K.), and directly sequenced using Big Dye terminators on an ABI 310 genetic analyzer (Perkin Elmer, Foster City, CA).

PCR amplification of genomic DNA and mutation detection After informed consent, genomic DNA was extracted from family members (see *Results*) by standard methods. DNA was not available from the deceased father of the proband. For direct amplification of the 13 plakophilin 1a coding exons of the *PKP1* gene from genomic DNA, primer pairs were positioned within the introns flanking the exonic sequences (**Table I**). For PCR, 200 ng of patient or control genomic DNA was added to a premix containing PCR buffer [67 mM Tris–HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.17 mg per ml bovine serum albumin (Sigma), and 10 mM 2-mercaptoethanol], 10 nmol of each dNTP, 20 pmol of each primer in a total volume of 50 μl. After an initial denaturation at 95°C for 2 min, 2.5 units of Taq polymerase (Promega) was added, followed by 35 cycles of 94°C for 10 s, annealing for 10 s, 72°C for 30 s, with a final incubation of 72°C for 5 min. The annealing temperatures for each primer pair are displayed in **Table I**. The PCR products were examined by 3% agarose gel electrophoresis, purified, and directly sequenced as above. Sequence variants were verified from genomic DNA by amplification of the specific exon followed by either restriction enzyme digestion or direct sequencing as displayed in **Table II**. Sequence alterations were examined in 50–100 control chromosomes.

Table I. Primers for amplification of plakophilin 1a exon sequences

Exon no.	cDNA[a]	5′ primer (sense)[b]	3′ primer (anti-sense)[b]	Annealing temp. (°C)	Product size (bp)
1	(–79)-201	gcccgcccgctgcac (–79)	ccccgccaatgggccagg (+65)	64	346
2	202–306	ccccaccttctcctccag (–140)	gatgagcctcaactcacggac (+130)	58	374
3	307–701	ccttccaggccaaacattctgg (–133)	gtccattacaccaggggc (+152)	62	680
4	702–846	tctggctctccagagggc (–120)	gcaagagaagccaactgc (+109)	58	374
5	847–1054	gttatcatgacctcacactgc (–158)	ggccagtgtcactaaactctc (+160)	58	526
6	1055–1232	gacaataggttctacagacca (–143)	tgccgggttgtctgttatgtt (+113)	58	434
7	1233–1347	accacaacttccaatctccac (–155)	tgagtctgtgctcctgtcttg (+104)	58	374
8	1348–1503	agtgtggtggtgcctgcg (–136)	gtgcaagatgaatggcctcta (+191)	58	483
9	1504–1680	gcctctgcaggcgcttcc (–135)	cagatcccagcatatgcc (+148)	62	460
10	1681–1834	ggaagccacaggctagct (–42)	aggctcccttctcctgtc (+157)	58	353
11	1835–2021	gggccacctggctcagag (–119)	gcctcccccgtccctggc (+137)	62	443
12	2022–2106	gctcctctcttttgcacacct (–141)	gacgctgtctttgtctctcag (+137)	58	363
13	2107–2213	aggccagttttcgccctg (–150)	cttgtgttgtccagagcc (+133)	58	390

[a]cDNA amplified in each fragment according to GenBank accession no. Z73678 relative to the translation initiation site.
[b]Within the intron, in relation to the intron/exon border (–1/+1), except for underlined numbers, which are within the exon relative to the translation start site.

Table II. Polymorphisms in the *PKP1* gene

Location	Nucleotide position[a]	Allelic codons		Detection	Allelic Frequency[b]	PIC Value
		Major	Minor			
Exon 3	586	ATA (Ile)	GTA (Val)	Sequencing	0.99/0.01 (78)	0.020
Intron 4	847–22	C	T	*Tsp*RI	0.77/0.23 (92)	0.291
Exon 5	918	GCC (Ala)	GCT (Ala)	*Sac*II	0.77/0.23 (92)	0.291
Intron 8	1503 + 22	C	T	*Fau*I	0.70/0.30 (106)	0.332
Exon 12	2050	CGG (Arg)	TGG (Trp)	*Msp*I	0.98/0.02 (100)	0.038

[a]According to GenBank accession no. Z73678 relative to the translation initiation site.
[b]Number of chromosomes tested in brackets.

Transmission electron microscopy and immunoelectron microscopy After informed consent, a biopsy from skin adjacent to an area of crusting was sampled and subdivided for transmission electron microscopy and immunolabeling studies. For electron microscopy, skin was fixed in 2% glutaraldehyde in sodium cacodylate buffer, followed by secondary fixation in 1% osmium tetroxide, and embedding in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM120 transmission electron microscope. For light microscopic immunolabeling studies, skin was freeze plunged into liquid propane and freeze substituted with lowicryl K11M (Chemische Werke Lowi, Waldkraiburg, Germany), as described previously (Haftek *et al*, 1994). One micron sections were labeled with primary antibodies to plakophilin 1 (PP1–5C2, undiluted, Cymbus Technology, Chandlers Ford, U.K.) or plakoglobin (PG5.1, diluted 1:40, Progen, Heidelberg, Germany) followed by goat anti-mouse IgG immunogold conjugate (1 nm particles; Auroprobe, Amersham, Little Chalfont, U.K.) and silver enhancement (IntenSE M kit, Amersham), as recommended by the manufacturer.

Figure 1. Clinical examination shows signs of abnormal keratinocyte adhesion and differentiation. Several peripherally spreading, circinate scaly plaques can be seen on the patient's legs. There are no bullae but the marginal skin is tender and slightly edematous. There is some spontaneous healing at the center of the lesions. Acral hyperkeratosis and a fissure on the heel are evident.

RESULTS

Clinical The patient was a 17 y old male, the third child of French parents of caucasian origin. Neither parent, the other siblings, or any other relatives had a history of skin, hair, or nail abnormalities. Within the first hours after birth, the patient developed a generalized erythema with skin blisters occurring at sites of mechanical trauma, although there was no mucosal involvement. His hair was very short, sparse, and fragile. Finger- and toe-nails were thickened and dystrophic or absent. Subsequently, skin fragility and dystrophic nails have persisted, but in addition, hyperkeratosis of the palms and soles has developed. Of note, affecting most of his skin are trauma-associated annular scaly hyperkeratotic crusty plaques (**Fig 1**). Many of these develop after scratching, particularly as mild generalized pruritus is an additional feature. The acral hyperkeratosis has been associated with painful cracking of the skin on the palms and soles, so disabling that for most of the time he has to use a wheelchair instead of walking. The patient remained bald up to the age of 6 y. Then, fragile curly scalp hair developed, although eyelashes and eyebrows have continued to be extremely sparse. There has been no evidence of dental abnormalities, nor any respiratory, gastrointestinal, genitourinary, ocular, or sweating problems. He has completed secondary education and is of normal intelligence. All hematologic and biochemical blood tests have appeared normal and there was no evidence of consanguinity within the family.

Genomic cloning of *PKP1* To initiate genomic cloning of *PKP1*, primers were positioned throughout the 2.7 kb of *PKP1* cDNA to identify introns that were then sequenced. Sequence analysis revealed that *PKP1* consisted of 15 exons spanning approximately 50 kb of genomic DNA (**Fig 2**). Plakophilin 1a is encoded for by the 3′ end of exon 1, exons 2–12, and the 5′ end of exon 13. The splice variant plakophilin 1b includes an additional 63 bp exon 6a. The amino-terminal domain of plakophilin 1a is encoded by exons 1–4, and the armadillo repeat units are encoded by exons 5–13 (**Fig 2**). The 5′ segment of exon 1, and the 3′ segment of exon 13 and the whole of exon 14 correspond to the 5′ and 3′ untranslated regions, respectively. The exons varied from

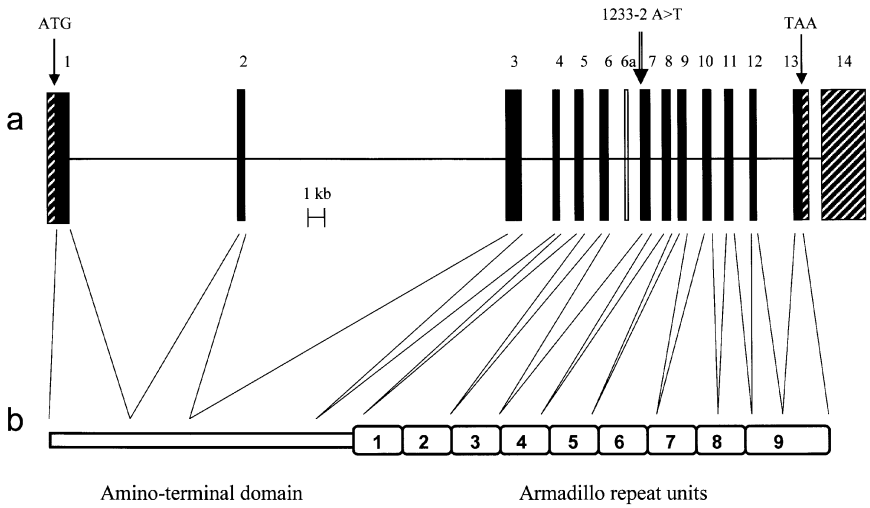

Figure 2. PCR amplification of genomic DNA establishes the intron–exon organization of *PKP1*. (*a*) Exons are represented by vertical boxes, introns by horizontal lines. The gene consists of 15 exons, 63–2920 bp in size, spanning approximately 50 kb of genomic DNA. Exon 6a is represented as an open box as it is not part of the plakophilin 1a transcript. The positions of the translation–initiation codon (ATG) in exon 1 and of the termination codon (TAA) in exon 13 are indicated. The homozygous mutation discovered in this study (1233–2 A→T) is indicated by the *arrow*. (*b*) Exons 1–4 of *PKP1* encode the amino terminus and exons 5–13 encode the armadillo repeat units.

63 bp (exon 6a) to ≈ 2.9 kb (exon 14) (**Fig 2**). The smallest intron was 348 bp (intron 10), whereas the largest was approximately 19 kb (intron 2). Examination of the exon–intron junctions revealed that the 5′ donor and 3′ acceptor splice sites of *PKP1* conform to the consensus ag/gt rule.

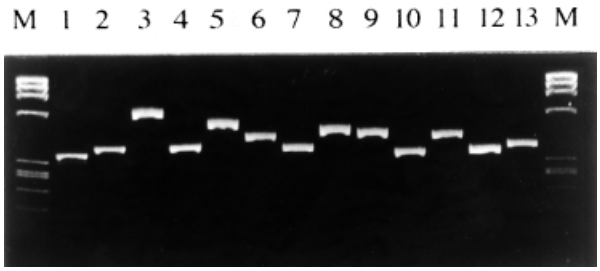

Figure 3. Agarose gel electrophoresis demonstrating amplification of the entire coding region of *PKP1*. PCR amplification of exons 1–13 and flanking introns using genomic DNA as template with the primer pairs as shown in **Table III**. The lanes M contain ØX174 *Hae*III molecular weight markers.

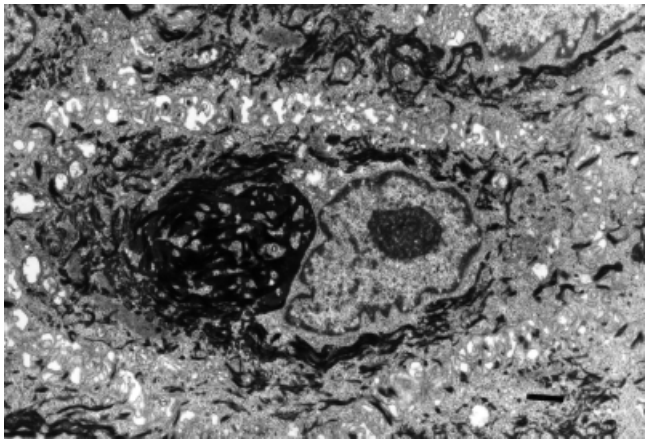

Figure 4. Transmission electron microscopy reveals poorly formed desmosomes and an abnormal keratin filament network. In this biopsy taken from normal appearing skin on the thigh at the periphery of a circinate lesion, few, small desmosomes are present. The most striking feature is the compacted, perinuclear arrangement of keratin filaments and the loss of attachment of the keratin filament network to the cell periphery. *Scale bar*: 1 μm.

Development of a PCR-based mutation detection strategy To facilitate identification of pathogenetic mutations in ectodermal dysplasia/skin fragility syndrome, we designed a strategy for detection of sequence variants in *PKP1*, consisting of PCR amplification of exons 1–13 directly by use of genomic DNA as a template, with primers based on intron-specific sequences followed by direct sequencing. To allow PCR amplification of exons directly from genomic DNA, balanced primer pairs were designed on the basis of sequences on the *PKP1* intron–exon regions. The primers were placed > 40 bp away from the intron–exon borders. The primer sequence information, the optimized amplification conditions, and the expected sizes of the PCR products are indicated in **Table I**. Agarose gel electrophoresis of the PCR products is shown in **Fig 3**.

Perturbations in desmosome morphology and complete absence of immunostaining for plakophilin 1 Ultrastructural assessment of the patient's skin revealed widening of intercellular spaces between adjacent keratinocytes. Most notably, the keratinocyte filament network throughout the spinous layer of the epidermis was abnormal, with poor connections to desmosomes at the cell periphery and a compacted clumped perinuclear appearance (**Fig 4**). Desmosomes appeared small and reduced in number throughout the epidermis. Immunolabeling of skin sections revealed a complete absence of immunostaining for plakophilin 1 in the patient's skin, but a normal pattern of labeling for plakoglobin (**Fig 5**).

Identification of a homozygous splice site mutation in PKP1 Direct sequencing of the proband's DNA revealed a homozygous A→T substitution at the acceptor −2 splice site of intron 6 designated 1233–2 A→T (**Fig 6*a***). As the identified acceptor splice site mutation does not change a restriction site other family members were screened for the mutation using direct sequencing of the exon 7 PCR product. This showed that the proband's two unaffected brothers, his mother, and maternal grandmother were all heterozygous carriers for the mutation 1233–2 A→T (**Fig 6*b***). Although cDNA was not available from the proband, the mutation is most likely to lead to aberrant splicing out of exon 7, with a subsequent premature termination codon (PTC) 154 bp downstream in exon 8. The mutation was not detected in direct sequencing of 100 control chromosomes from ethnically matched individuals. Elucidation of mutations in this family brings the total number of patients with mutations identified in both alleles of the *PKP1* gene to three (**Table III**). During screening for pathogenic mutations several nonpathogenic sequence

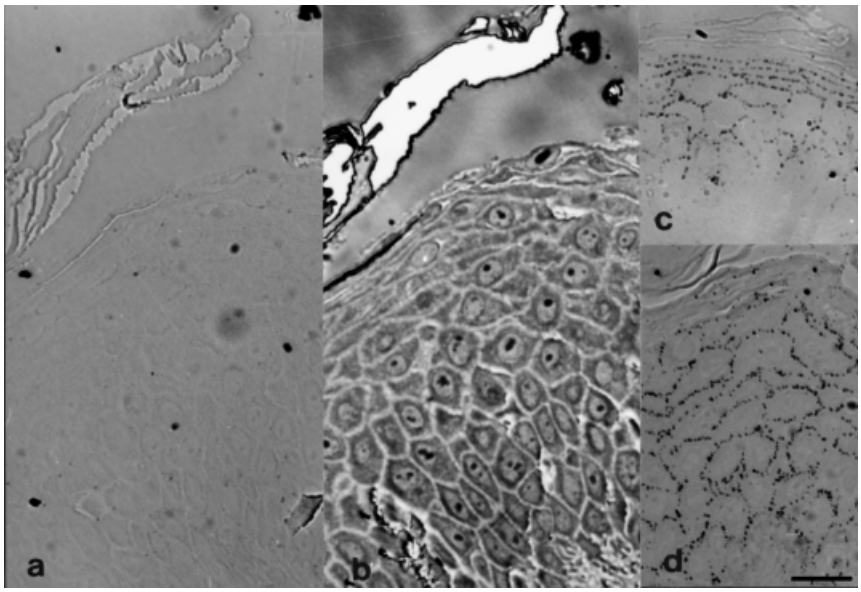

Figure 5. Light microscopic immunogold-silver enhancement labeling demonstrates complete ablation of plakophilin 1. There is no specific labeling with anti-*PKP1* antibody of plastic embedded perilesional epidermis of the patient (*a*). A few large silver granules seen on the section testify to a long-lasting amplification. The counterstained semithin section in (*b*), shows the topography of the acanthotic epidermis labeled in (*a*). Under the same experimental conditions, the anti-*PKP1* antibody labeled normal human epidermis in an intercellular punctate pattern (*c*). The patient's *PKP1* unreactive epidermis showed normal expression of plakoglobin (*d*). Note that the epidermis in the patient's skin is thicker than the control skin (compare *a*, *b* and *d* with *c*). *Scale bar*: 20 μm.

polymorphisms were identified, the genotypes of which are in Hardy–Weinberg equilibrium: these are detailed in **Table II**.

DISCUSSION

We have determined that the *PKP1* gene consists of 15 exons and spans approximately 50 kb of genomic DNA. Comparing the genomic structure of *PKP1* with the preliminary data obtained for both plakophilin 2 (*PKP2*) and plakophilin 3 (*PKP3*) (Schmidt *et al*, 1999) it is clear that there is considerable exon border homology within the armadillo repeat units of all three genes; however, it remains to be established whether this also applies to the genomic organization of the corresponding amino-terminal domains. The first demonstration of an inherited abnormality in *PKP1* consisted of a report of a 6 y old male with an atypical ectodermal dysplasia/skin fragility syndrome that caused blistering of the palms and soles, sparse hair, and thickened dystrophic nails (McGrath *et al*, 1997). The second report comprised an 18 mo old child with near-identical clinical features (McGrath *et al*, 1999). Both cases had immunohistochemical evidence of complete ablation of *PKP1*, with supportive molecular data. The present case, with similar laboratory findings, details an older 17 y old male that allows for further assessment of the phenotypic consequences of *PKP1* mutations. Specifically, the earlier reports demonstrated a complete lack of scalp hair; however, in this case the proband started to regrow some scalp hair (as well as very sparse eyebrows and eyelashes) from the age of 6 y, although the hair remained fragile. In addition, it is clear that *PKP1* ablation results in a disabling, diffuse hyperkeratosis of the palms and soles with marked fissuring of the skin. Of note, the 17 y old was often confined to a wheelchair because of the severe discomfort. This information is relevant to genetic counseling of the other affected patients. The initial *PKP1* mutation reports suggested a possible impairment of sweating in the affected children, but this was not a feature in the 17 y old. Furthermore, aside from the hair and nail dystrophy, there did not appear to be any other significant epithelial abnormalities. Given the known tissue distribution of plakophilin 1 (Moll *et al*, 1997), it is possible that the relatively restricted phenotype in patients with *PKP1* ablation may reflect compensation in other tissues by additional plakophilins or other components of the desmosomal plaque in limiting desmosomal disruption.

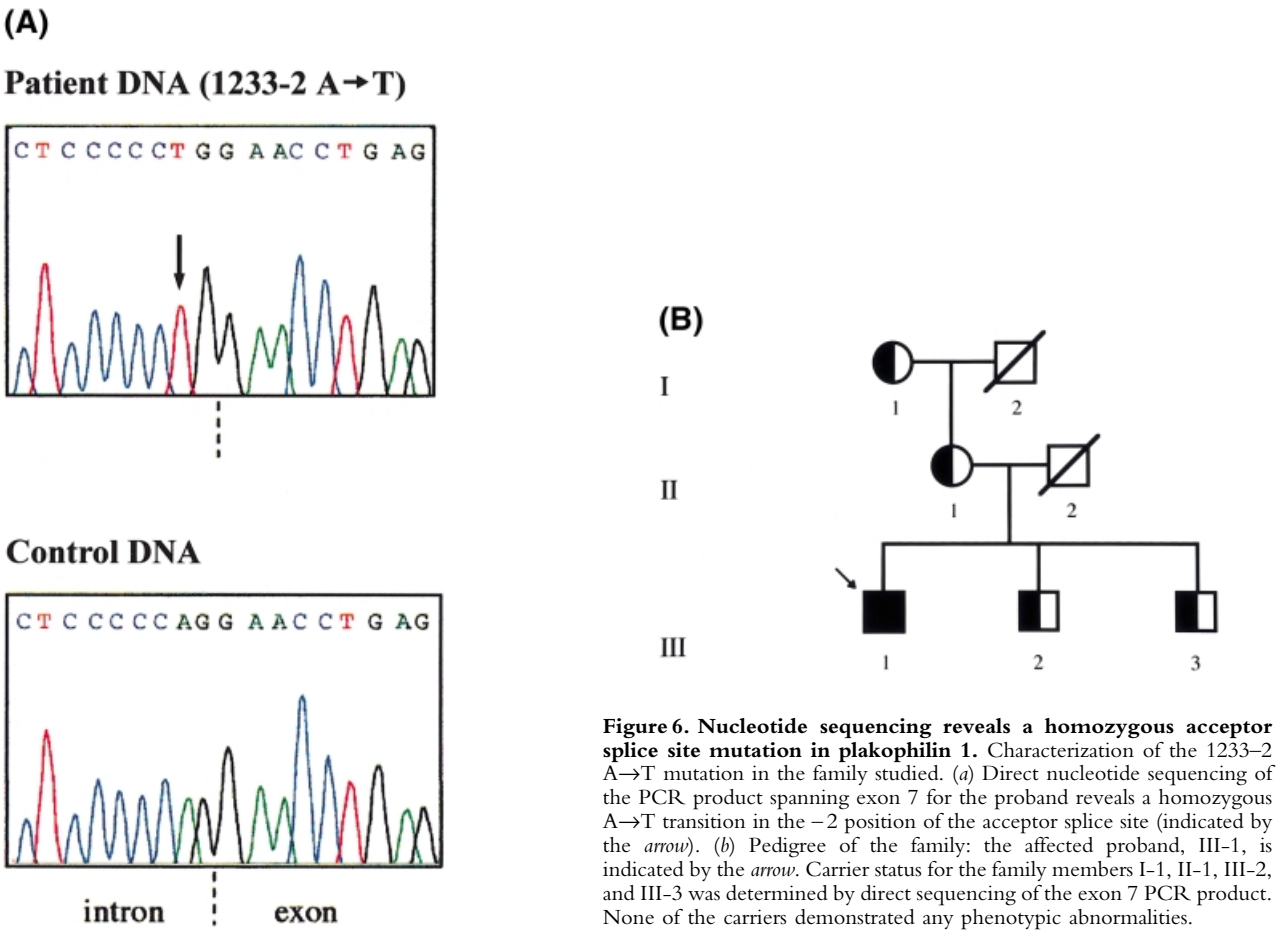

Figure 6. Nucleotide sequencing reveals a homozygous acceptor splice site mutation in plakophilin 1. Characterization of the 1233–2 A→T mutation in the family studied. (*a*) Direct nucleotide sequencing of the PCR product spanning exon 7 for the proband reveals a homozygous A→T transition in the −2 position of the acceptor splice site (indicated by the *arrow*). (*b*) Pedigree of the family: the affected proband, III-1, is indicated by the *arrow*. Carrier status for the family members I-1, II-1, III-2, and III-3 was determined by direct sequencing of the exon 7 PCR product. None of the carriers demonstrated any phenotypic abnormalities.

Table III. List of *PKP1* mutations in patients with ectodermal dysplasia/skin fragility syndrome

Patient	Genetic status	Mutations[a]	Inheritance	Verification	Consequence	Reference
1	Compound heterozygote	Q304X	Paternal	*Bfa*1	PTC	McGrath *et al* 1997
		1132ins28	Maternal	Electophoresis	PTC	
2	Compound heterozygote	203 1 G→A	Paternal	*Bfa*1	PTC	McGrath *et al* 1999
		Y71X	Maternal	*Fok*1	PTC	
3	Homozygote	1233 2 A→T	Paternal	Sequencing	PTC	This study
		1233 2 A→T	Maternal	Sequencing	PTC	

[a]According to GenBank accession no. Z73678 relative to the translation initiation site.

Unfortunately, cDNA was unavailable for this study, and we are therefore unable to confirm the mechanism of splicing within the mutant plakophilin 1 transcript. In general, splice site mutations can result in either: (i) the skipping of an exon; (ii) the inclusion of an intron; or (iii) the use of a cryptic splice site (Berget, 1995). The most frequently observed result of a splicing mutation is exon skipping (Nakai and Sakamoto, 1994), and indeed the acceptor splice site mutation characterized in this study would be expected to result in the skipping of exon 7 leading to a frameshift and a subsequent PTC in exon 8 (Maquat, 1996). As a result of non-sense mediated mRNA decay very low levels of plakophilin 1 protein would be expected and this was confirmed in our patient by the complete lack of anti-*PKP1* staining in perilesional epidermis.

This study also identified four family members who were heterozygous carriers of the splice site mutation. None of these individuals had any clinical symptoms or signs of cutaneous disease. Likewise, heterozygous carriers of the previously reported *PKP1* mutations have not been found to have any epithelial or epidermal pathology. Thus, haploinsufficiency for *PKP1* does not appear to result in any clinical disorder. These observations are in stark contrast to other recent studies that have identified haploinsufficiency mutations in other structural proteins of desmosomes. Specifically, it has been shown that the autosomal dominant skin disorder, striate palmoplantar keratoderma, is a heterogeneous disease resulting from haploinsufficiency of the desmosomal proteins desmoplakin I (Armstrong *et al*, 1999; Whittock *et al*, 1999) or desmoglein 1 (Rickman *et al*, 1999). Considerable work has now been done using expression studies and yeast two-hybrid systems to determine how the components of desmosomes associate and it is therefore important to understand how these mutations have their effect on the integrity of the desmosome. In dividing epidermal cells, desmoplakin and plakoglobin anchor to desmosomal cadherins and to each other creating an organized matrix that binds to the keratin molecules of the intermediate filaments (Smith and Fuchs, 1998). For this purpose, plakoglobin contains a series of hydrophobic amino acid residues within armadillo repeats 1–3, that bind to the carboxy terminus of the cytoplasmic domain of desmosomal cadherins (Mathur *et al*, 1994; Troyanovsky *et al*, 1994; Wahl *et al*, 1996; Chitaev *et al*, 1998; Smith and Fuchs, 1998). It appears that plakoglobin functions by potentiating heterodimer formation of desmogleins and desmocollins, accumulation of desmosomal cadherin heterodimers to cell–cell contact sites or perhaps desmosomal cadherin stability (Marcozzi *et al*, 1998). During differentiation of epidermal cells, however, the amino-terminal globular domain of plakophilin 1 binds to desmoplakin (Smith and Fuchs, 1998; Kowalczyk *et al*, 1999; Hatzfeld *et al*, 2000) improving lateral interactions between desmoplakin molecules, which in turn bind the keratin intermediate filaments. It can therefore be concluded that the epidermal lesions seen in patients with plakophilin 1 ablation are a consequence of the loss of desmosome integrity resulting from a decrease in binding sites for desmoplakin and intermediate filaments (Kowalczyk *et al*, 1999). In addition to its desmosomal and nuclear localization (Schmidt *et al*, 1997) plakophilin 1 has also been found to be distributed along actin filaments, an association that is mediated by its central arm repeat domain (Hatzfeld *et al*, 2000). This suggests that plakophilin 1 may also be involved in regulating actin filament organization as well as desmosome stabilization (Hatzfeld *et al*, 2000).

In summary, we have elucidated the genomic organization of *PKP1* and have developed a strategy for the detection of sequence variants in this gene. Utilizing this strategy, we have identified a number of polymorphisms and have delineated a new mutation in a family with ectodermal dysplasia/skin fragility syndrome. Collectively, the results underscore the crucial part plakophilin 1a plays in providing integrity to the desmosomal attachment within the epidermis and its appendages.

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